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Supplementary Materials for

Assembly of embryonic and extra-embryonic stem cells to mimic embryogenesis in vitro

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This PDF file includes:

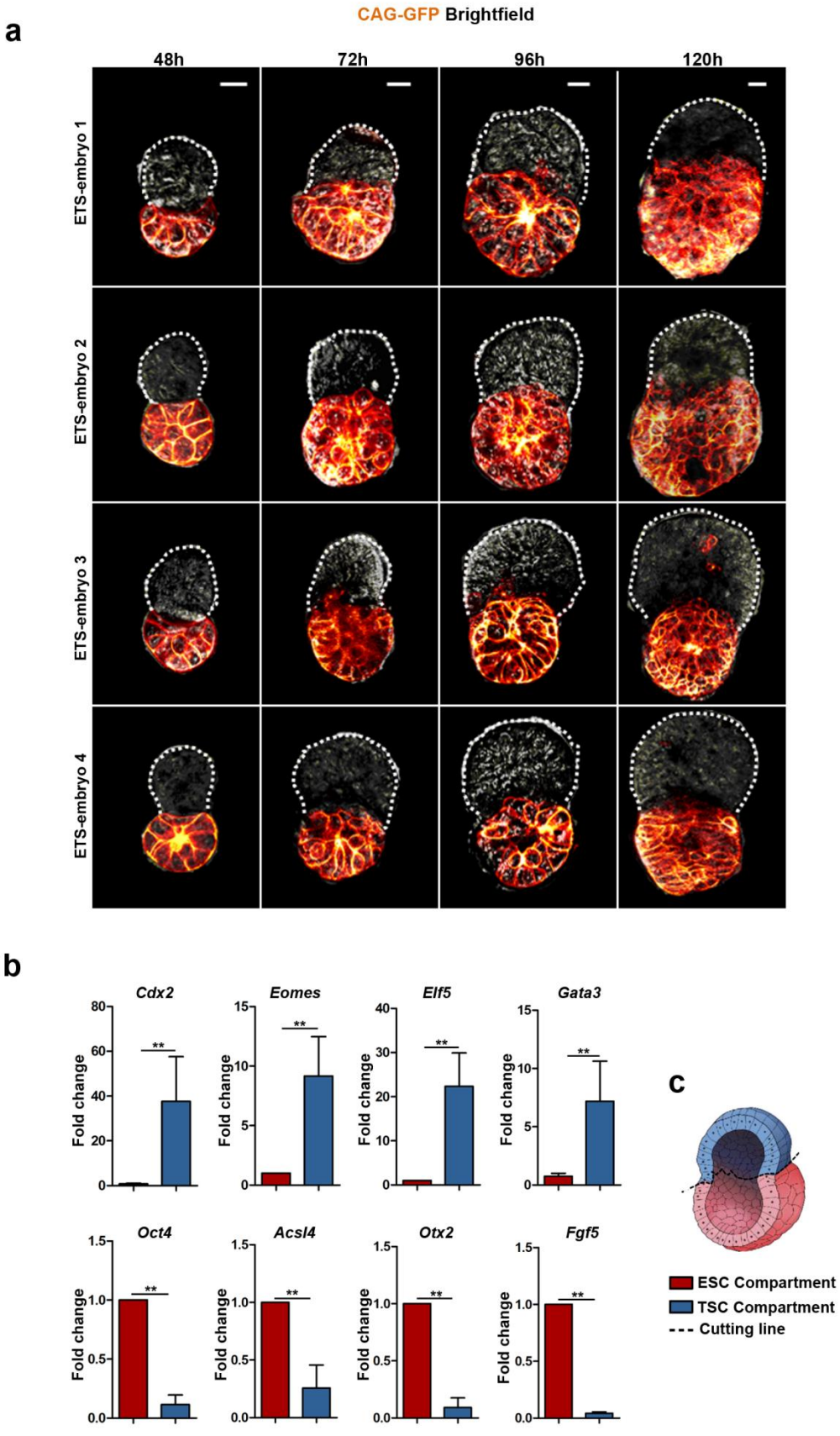
a
CAG-GFP Brightfield
48h
72h
96h
120h
ETS-embryo 1
ETS-embryo 2
ETS-embryo 3
ETS-embryo 4
b
Cdx2
Eomes
Elf5
Gata3
Oct4
Acsl4
Otx2
Fgf5
Fold change
**
c
ESC Compartment
TSC Compartment
Cutting line

Fig. S1. a. Confocal time-lapse images of 4 representative ETS-embryos at indicated times in which CAG-GFP ESCs (orange) labels the embryonic compartment. Brightfield was false-coloured with the 'edges' "Look-up-table" function in Fiji software. White dotted line marks the outside of the TSC compartment for clarity. Bar=20μm. **b.** q-RT-PCR analysis of ExE markers (*Cdx2*, *Eomes*, *Elf5*, *Gata3*) and epiblast markers (*Oct4*, *Acsl4*, *Otx2*, *Fgf5*) in ESC and TSC derived compartments of ETS-embryos cultured for 96 hours. Student's t-test, $P<0.05$. n=4 biological replicates. Error bars= SEM. **c**. Schematic representation of a "ETS-embryo" to illustrate how ESC (red) and TSC (blue) compartments were isolated for RT-qPCR analysis.

Figure S2

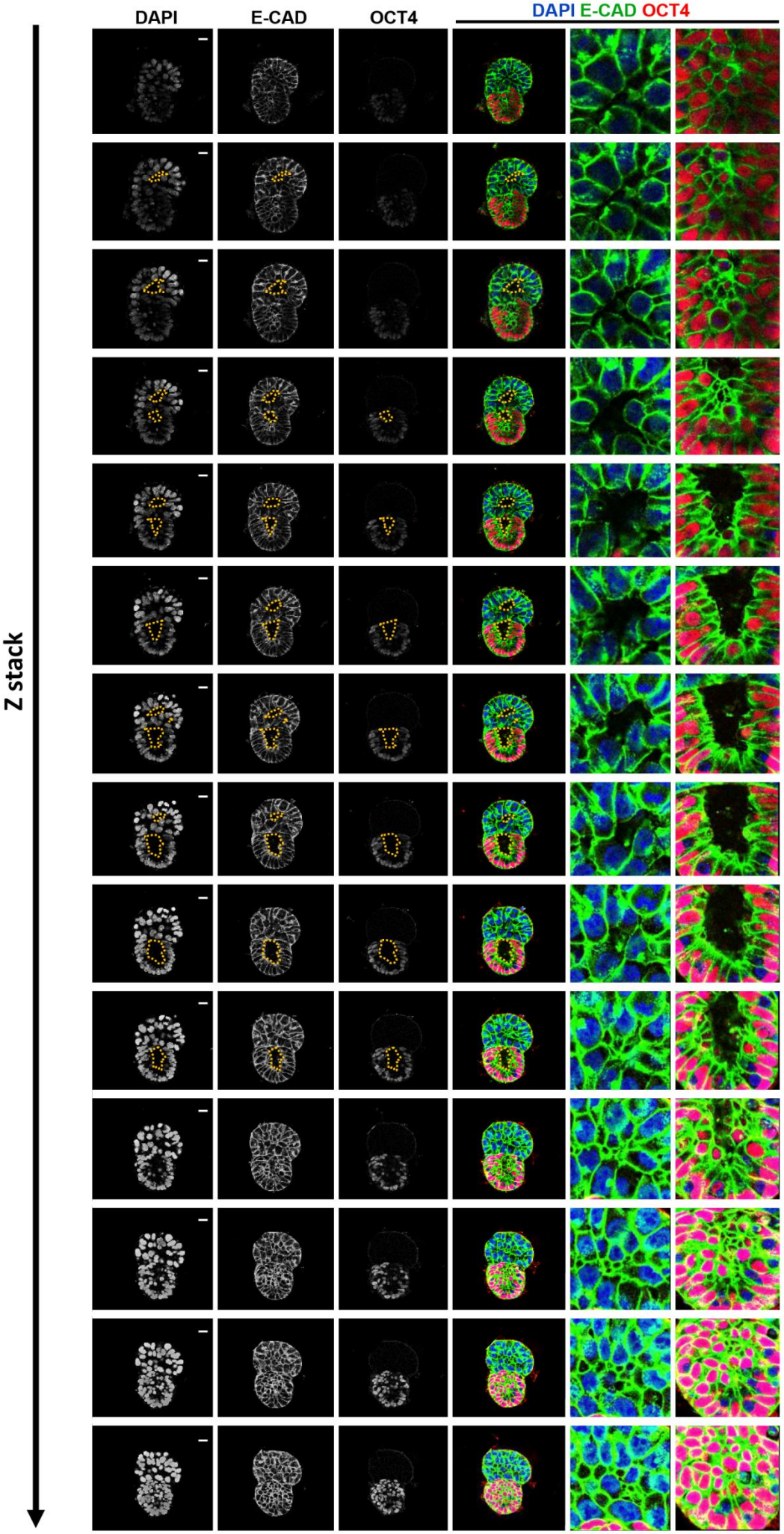

Fig. S2. A montage of a complete Z-stack through a representative ETS-embryo to illustrate scoring of cavities (outlined with yellow dotted lines and shown in zoomed images in right-most two columns). Red, Oct4; Green, E-cadherin; Blue, DNA. Scale bar=20μm.

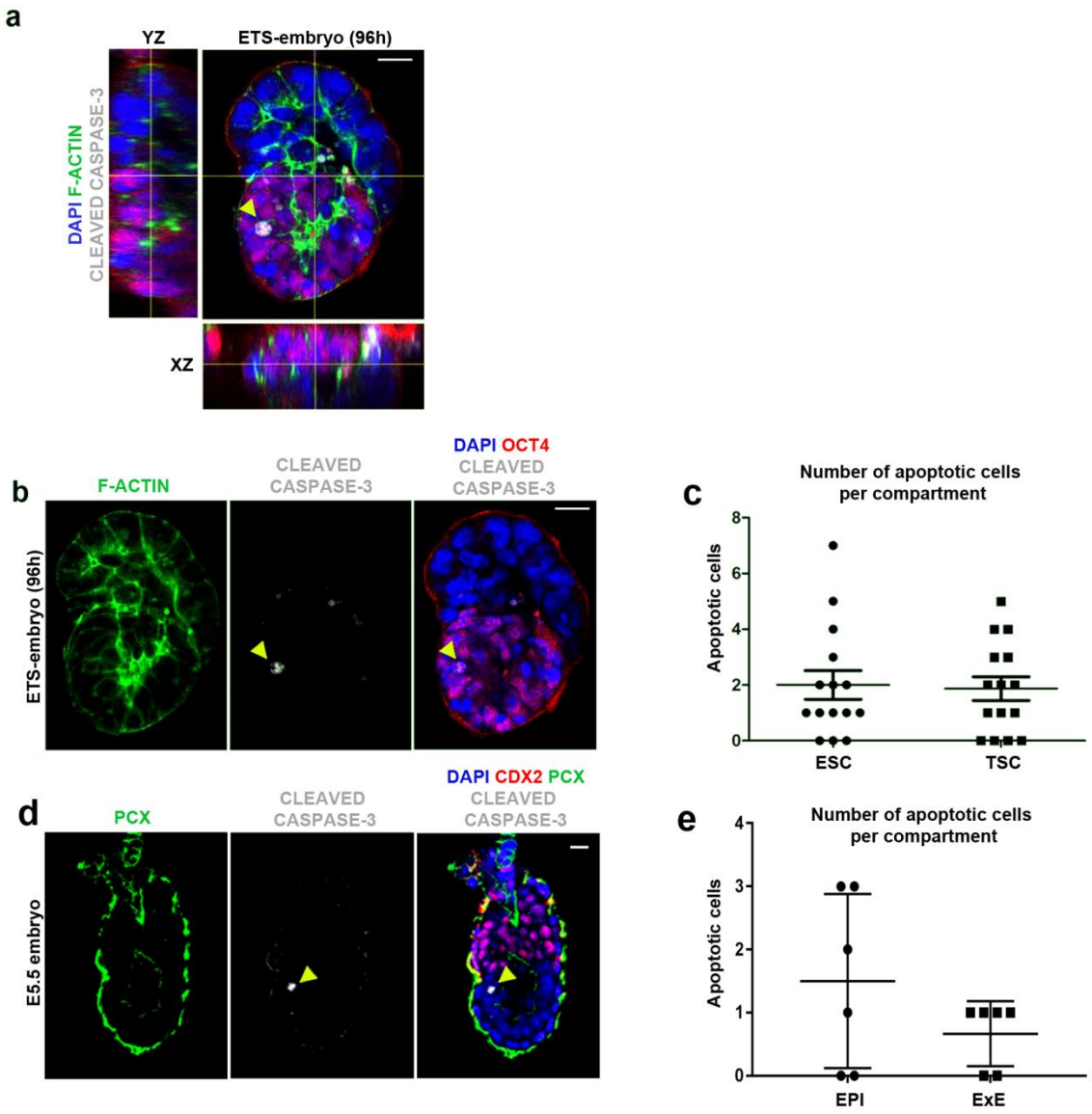

Fig. S3. a-b. ETS-embryo stained to reveal: Oct4, red; F-actin, green; DNA, blue; Cleaved-caspase-3, grey. Bar=20µm. Yellow arrowhead indicates a dying cell. XZ and YZ orthogonal views are also shown in (**a**). **c.** Quantification of dying cells in ESC and TSC compartments. n=15. **d.** E5.5 embryo recovered from mother and stained to reveal: Cdx2, red; PCX, green; DNA, blue; Cleaved-caspase-3, grey. Bar=20µm. Yellow arrowhead indicates a dying cell. **e.** Quantification of dying cells in the embryonic (EPI) and extra-embryonic (ExE) compartments of the embryo. n=6.

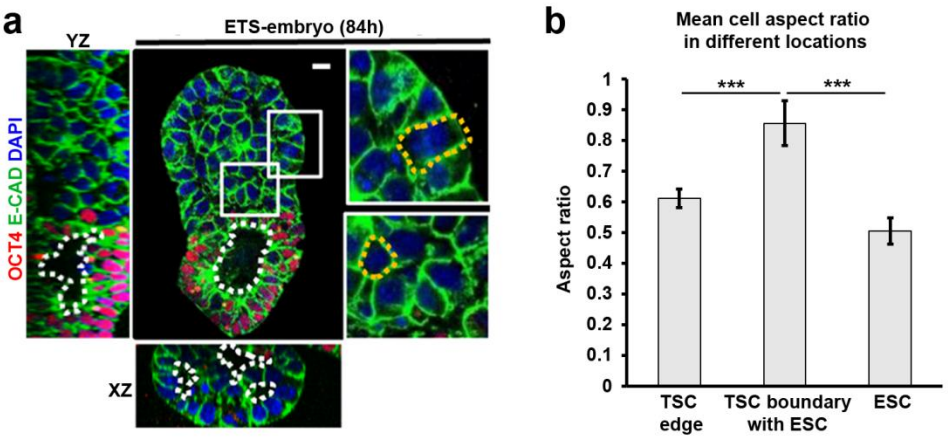

Fig. S4. a. ETS-embryo at 84 hours stained to reveal Oct4, red; E-cadherin, green; DNA, blue. XZ and YZ orthogonal views also shown. White dotted lines highlight cavities. Insets highlight individual cells outlined in yellow dotted lines to indicate cell shape. Bar= 20μm. n=30, 3 separate experiments. **b.** Mean cell aspect ratio (width of cell divided by length) is significantly different between ESC and TSC compartments of ETS-embryos at 84 hours of development. ANOVA test, $P<0.001$, n=30 per group, 3 separate experiments. Error bars= SEM.

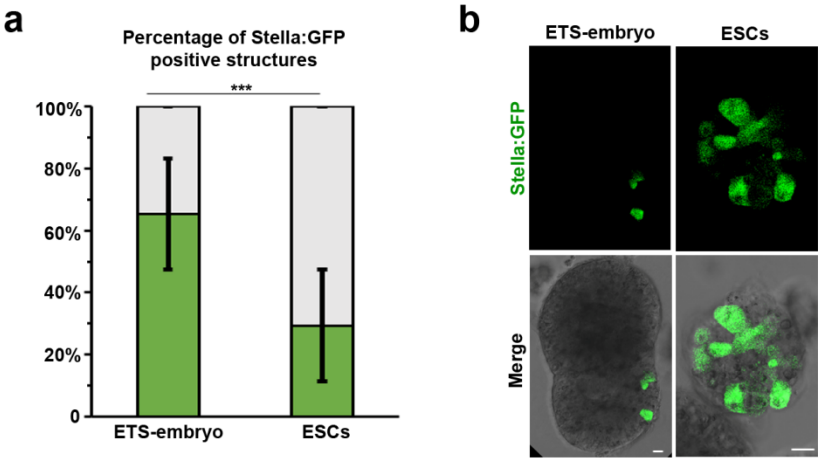

Fig.S5 a. Proportion of ETS-embryos expressing Stella:GFP at 120 hours is significantly higher in comparison to ESCs-alone structures. Fisher's exact test, $P<0.001$, n=80: 40 ETS-embryos and 40 ESC-alone structures counted in 2 experiments. Error bars=SEM. **b.** Stella:GFP-expressing ESCs (green) growing alone (right) or as part of a ETS-embryo (left) in Matrigel. Bar=20µm. n=40 "ETS-embryos", 2 experiments; n=20 ESC-alone structures, 2 experiments.

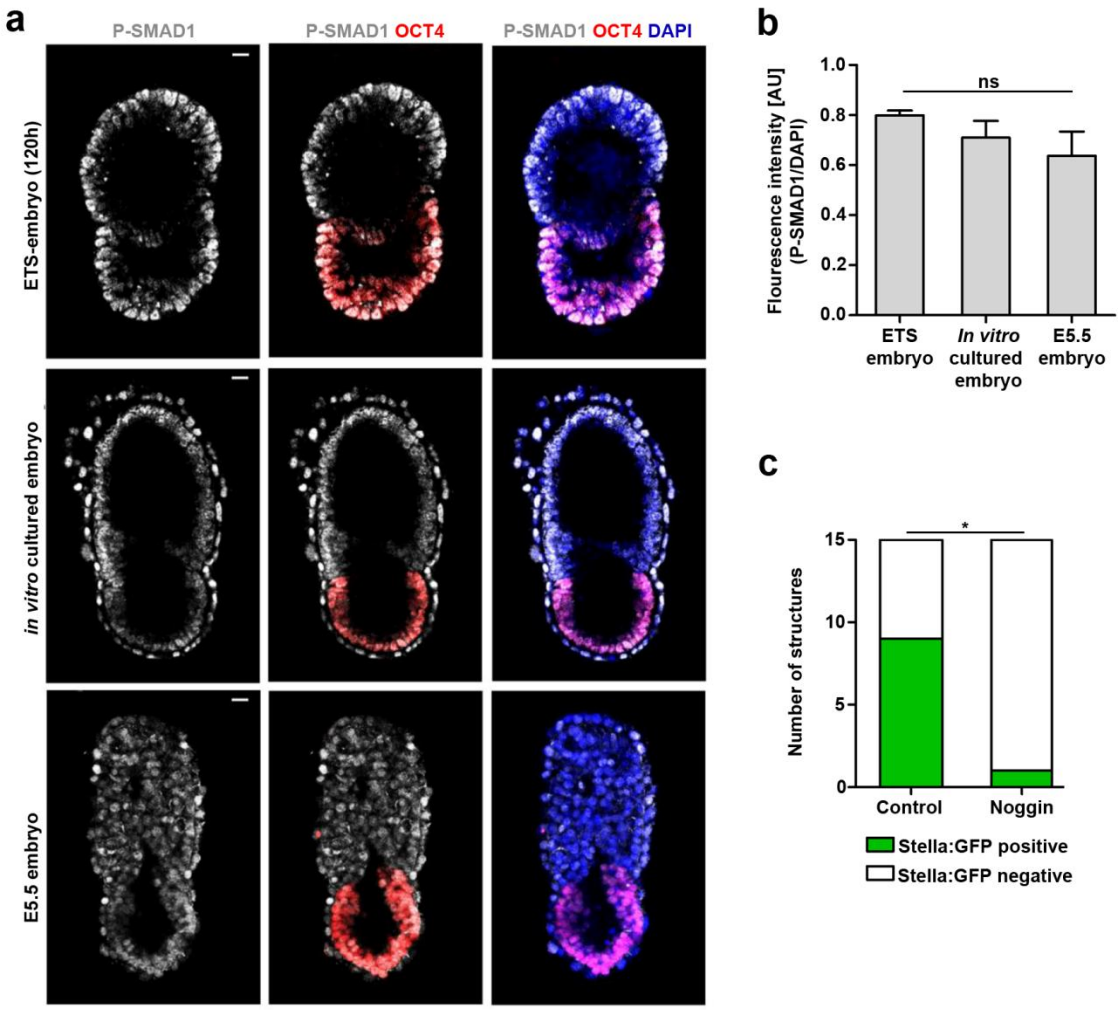

Fig. S6 a. Comparison of P-SMAD1 expression in ETS-embryo*;* an *in vitro* cultured embryo and an embryo recovered from the mother at E5.5. P-SMAD1, grey; Oct4, red; DNA blue. Bar= 20µm. **b.** P-SMAD1 immunofluorescence intensity quantified in ETS-embryos, natural embryos recovered from the mother at E5.5 and *in* vitro cultured embryos at egg cylinder stage. P-SMAD1 intensity was normalised to the DNA-channel (DAPI, blue) and a mean average was taken. ANOVA test, not significant, n=4 per group**.** Error Bars= SEM. **c.** Quantification of the number of ETS-embryos with Stella:GFP expression at the boundary between ESC and TSC compartments after 120 hours in culture in control conditions and in the presence of Noggin. Count data are presented as a bar chart, and a contingency table was used to perform the statistical test. n=15 per group, 3 separate experiments. Fisher's exact test, $P<0.05$.

Supplementary table 1- Antibodies used in this study

Antibody (species)	Vendor	Number	Dilution
Oct 3/4 (mouse)	Santa cruz	sc-5279	1:200
Tbr2/Eomes (rabbit)	abcam	ab23345	1:400
aPKC (rabbit)	Santa cruz	sc-17781	1:200
Podocalyxin (rat)	R&D systems	MAB1556	1:400
Brachyury/T (goat)	Santa cruz	sc-17745	1:50
GFP (rat)	Nacalai biochemicals	04404-84	1:2000
Tfap2c (rabbit)	Santa cruz	sc-8977	1:200
Laminin (rabbit)	Sigma	L9393	1:400
Cdx2 (mouse)	Launch diagnostics	MU392-UC	1:200
E-cadherin (rat)	Life Technologies (Thermofisher scientific)	13-1900	1:400
Phospho-SMAD2/3	Cell signalling technologies	8828P	1:200
Phospho-SMAD 1/5/9	Cell signalling technologies	13820P	1:200
Gata4 (Goat)	Santa cruz	sc-1237	1:200
Cleaved caspase-3 (rabbit)	Cell signalling technologies	#9664	1:200
F-actin (Phalloidin 488)	Life Technologies (Thermofisher scientific)	A12379	1:1000
Alexa 488 (Donkey anti-rat)	Life Technologies (Thermofisher scientific)	A21208	1:500
Alexa 568 (Donkey anti-mouse)	Life Technologies (Thermofisher scientific)	A10037	1:500
Alexa 647 (Donkey anti-rabbit)	Life Technologies (Thermofisher scientific)	A31573	1:500
Alexa 647 (Donkey anti-goat)	Life Technologies (Thermofisher scientific)	A21447	1:500

Supplementary table 2- qPCR primers used in this study

Gene	Forward (5' to 3')	Reverse (5' to 3')
Prdm14	ACAGCCAAGCAATTTGCACTAC	TTACCTGGCATTTTCATTGCTC
Stella	AGGCTCGAAGGAAATGAGTTTG	TCCTAATTCTTCCCGATTTTCG
Nanos3	CACTACGGCCTAGGAGCTTGG	TGATCGCTGACAAGACTGTGGC
Ddx4	GCAGTGTTGTAACGTCAGCATTTC	TTCTTCTGTTCTTCCTCCCAACC
Mixl1	GACAGACCATGTACCCAGAC	GCTTCAAACACCTAGCTTCAG
Hand1	ACGTGCTGGCCAAGGATGCA	TGGTTTAGCTCCAGCGCCCA
GAPDH	CGTATTGGGCGCCTGGTCAC	ATGATGACCCTTTTGGCTCC
Dnmt3b	CTCGCAAGGTGTGGGCTTTTGTAAC	CTGGGCATCTGTCATCTTTGCACC
Tfap2c	TGCCCACGTCACTCTCCTCA	TCCGTCCCCCAAGATGTGGT
T	GCTGGATTACATGGTCCCAAG	GGCACTTCAGAAATCGGAGGG
Blimp1	CGGAAAGCAACCCAAAGCAATAC	CCTCGGAACCATAGGAAACATTC
Wnt3	CAAGCACAACAATGAAGCAGGC	TCGGGACTCACGGTGTTTCTC
Axin1	ACGGTACAACGAAGCAGAGAGCT	CGGATCTCCTTTGGCATTCGGTAA
Pou3f1	TTCAAGCAACGACGCATCAA	TGCGAGAACACGTTACCGTAGA
Oct4	GATGCTGTGAGCCAAGGCAAG	GGCTCCTGATCAACAGCATCAC
Slc7a3	TTCTGGCCGAGTTGTCTATGTTTG	AGTGCGGTTCTGTGGCTGTCTC
Utf1	GGATGTCCCGGTGACTACGTCTG	GGCGGATCTGGTTATCGAAGGGT
Cdx2	AGTGAGCTGGCTGCCACACT	GCTGCTGCTGCTTCTTCTTGA
Eomes	TCGCTGTGACGGCCTACCAA	AGGGGAATCCGTGGGAGATGGA
Elf5	ATTCGCTCGCAAGGTTACTCC	GGATGCCACAGTTCTCTTCAGG
Gata3	GGGTTCGGATGTAAGTCGAG	CCACAGTGGGGTAGAGGTTG
Otx2	TATCTAAAGCAACCGCCTTACG	GCCCTAGTAAATGTCGTCCTCTC
Acsl4	CCTGAGGGGCTTGAAATTC	GTTGGTCTACTTGGAGGAACG
Fgf5	AACTCCATGCAAGTGCCAAAT	CGGACGCATAGGTATTATAGCTG